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L7 ANSWER 1 OF 7 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1998:129057 HCAPLUS

DOCUMENT NUMBER: 128:240002

TITLE: Analysis of gene expression patterns in small amounts of human ventricular myocardium by a multiplex RNase protection assay

AUTHOR(S): Mittmann, Clemens; Munstermann, Ursula; Weil, Joachim; Bohm, Michael; Herzig, Stefan; Nienaber, Christoph; Eschenhagen, Thomas

CORPORATE SOURCE: Pharmakologisches Institut, Universitats-Krankenhaus Eppendorf, Hamburg, D-20246, Germany

SOURCE: Journal of Molecular Medicine (Berlin) (1998), 76(2), 133-140
CODEN: JMLME8; ISSN: 0946-2716

PUBLISHER: Springer-Verlag

DOCUMENT TYPE: Journal

LANGUAGE: English

AB End-stage **human** heart failure is assocd. with changes in expression of steady-state mRNA (mRNA) levels. These changes correspond to alterations in protein levels and myocardial function and may have clin. implications regarding etiol., clin. state, or prognosis. However, anal. of mRNA levels in endomyocardial biopsies can be accomplished only by the quant. polymerase chain reaction, which is difficult to standardize. The aim of the study was to evaluate whether the RNase protection assay is applicable to measure mRNAs of multiple genes simultaneously in small amts. of ventricular myocardium comparable to myocardial biopsies. Total RNA was prepd. from left ventricular myocardium from terminally failing hearts with idiopathic (n=9) or ischemic cardiomyopathy (n=7) and from nonfailing control hearts (n=10). mRNA was measured by an optimized RNase protection assay for the .beta.1-adrenoceptor, the stimulatory G protein .alpha.-subunit (Gs.alpha.), phospholamban, the calcium **ATPase** of the sarco-plasmic reticulum (SERCA), .beta.-**myosin heavy chain** (.beta.-MHC), and the atrial natriuretic peptide (ANP). We extd. 10.7+-2.1 .mu.g total RNA from three myocardial biopsies taken in vitro. All of the six genes were measurable in duplicate in a total of 7 .mu.g RNA. MRNAs of .beta.1-adrenoceptor, phospholamban, and SERCA were lower in failing than in nonfailing myocardium by 50%, 33%, and 42% resp., whereas .beta.-MHC and Gs.alpha. mRNAs were unchanged. mRNA of ANP was expressed at high levels only in the failing myocardium, providing a highly specific and sensitive marker for discriminating nonfailing and failing hearts. A direct comparison with ANP and Gs.alpha. levels obtained by Northern blot anal. with 7.5 .mu.g total RNA showed a good correlation between the two methods. The RNase protection assay is thus a suitable method for simultaneous measurements of multiple mRNA levels in **human** myocardial biopsies. Changes in mRNA levels closely reflected those identified by other methods using larger amts. of RNA. Increased myocardial ANP mRNA levels detd. by the RNase protection assay may serve as a mol. marker of heart failure.

L7 ANSWER 2 OF 7 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1997:219452 HCAPLUS

DOCUMENT NUMBER: 126:302815

TITLE: Novel characteristics of a myosin isolated from mammalian retinal pigment epithelial and endothelial cells

AUTHOR(S): Alliegro, Mark C.; Linz, Laura A.

CORPORATE SOURCE: Department of Anatomy, Louisiana State University Medical Center, New Orleans, LA, 70112, USA

SOURCE: Journal of Biological Chemistry (1997), 272(13), 8759-8763
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We have isolated a novel, high Mr protein from **human** retinal

pigment epithelial cells and endothelial cells by affinity chromatog. on Sepharose 4B. Two polypeptides are present on SDS-gels on the 8 M urea eluent with apparent mol. mass of .apprx.210 and 47 kDa. In the absence of diethiothreitol, the two polypeptides migrate as one protein band with an apparent mol. mass of .apprx.550 kDa. "Piglet", as this mol. is tentatively named, is present in retinal pigment epithelial and endothelial cells of several species, but could not be detected in the nonepithelial cells we examd. Immunofluorescent localization using an antibody to the 210-kDa polypeptide revealed a filamentous network in the cytoplasm of cultured cells. This antibody was used to identify a **cDNA** for piglet in a bovine aortic endothelial cell expression library. Sequence data indicate a high degree of identity with non-muscle myosin II heavy chain. We subsequently found that piglet had an actin-activated **ATPase** activity, colocalized with actin in cells, and reacted on Western blots with a pan-non-muscle myosin II heavy chain antiserum. The protein was also recognized by antibodies specific for **myosin heavy chain** isoform A, but did not react with anti-isoform B antibodies. Although piglet has several features in common with known forms of non-muscle myosin II, the distinctly unconventional features it displays suggest that it is a novel myosin.

L7 ANSWER 3 OF 7 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1996:612997 HCAPLUS

DOCUMENT NUMBER: 125:267364

TITLE: Muscle-specific and inducible expression of 293-base pair .beta.-myosin heavy chain promoter in transgenic mice

AUTHOR(S): Wiedenman, Jennifer L.; Tsika, Gretchen L.; Gao, Liying; McCarthy, John J.; Rivera-Rivera, Ilia D.; Vyas, Dharmesh; Sheriff-Carter, Katrina; Tsika, Richard W.

CORPORATE SOURCE: Mol. Integrative Physiol., Univ. Illinois, Urbana-Champaign, IL, 61801, USA

SOURCE: American Journal of Physiology (1996), 271(3, Pt. 2), R688-R695
CODEN: AJPHAP; ISSN: 0002-9513

PUBLISHER: American Physiological Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The **DNA** regulatory element(s) involved in .beta.-**myosin heavy chain** (.beta.-MHC) induction by the physiol. stimulus of mech. overload have not been identified as yet. To delineate regulatory sequences that are required for mech. overload induction of the .beta.-MHC gene, transgenic mouse lines were generated that harbor transgenes contg. serial deletions of the **human** .beta.-MHC promoter to **nucleotides** -293 (.beta.293), -201 (.beta.201), and -141 (.beta.141) from the transcription start site (+1). Mech. overloaded adult plantaris and soleus muscles contained 11- and 1.9-fold increases, resp., in endogenous .beta.-MHC-specific mRNA transcripts (Northern blot) compared with sham-operated controls. Expression assays (chloramphenicol acetyltransferase specific activity) revealed that only transgene .beta.293 expression was muscle specific in both fetal and adult mice and was induced in the plantaris (10- to 27-fold) and soleus (2- to 2.5-fold) muscles by mech. overload. Histochem. staining for myosin **ATPase** activity revealed a fiber-type transition of type II to type I in the overloaded plantaris and soleus muscles. These transgenic data suggest that sequences located between **nucleotides** -293 and +120 may be sufficient to regulate the endogenous .beta.-MHC gene in response to developmental signals and to the physiol. signals generated by mech. overload in fast- and slow-twitch muscles.

L7 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1994:554628 HCAPLUS

DOCUMENT NUMBER: 121:154628

TITLE: Gene expression of the cardiac Na+-Ca2+ exchanger in end-stage human heart failure

AUTHOR(S): Studer, Roland; Reinecke, Hans; Bilger, Johannes; Eschenhagen, Thomas; Boehm, Michael; Hasenfuss, Gerd;

CORPORATE SOURCE: Just, Hanjoerg; Holtz, Juergen; Drexler, Helmut
Medizinische Klinik III, Universitaet Freiburg,
Freiburg, 79106, Germany
SOURCE: Circulation Research (1994), 75(3), 443-53
CODEN: CIRUAL; ISSN: 0009-7330

DOCUMENT TYPE: Journal
LANGUAGE: English

AB The regulation of cytosolic Ca^{2+} concn. during excitation-contraction coupling is altered in the failing **human** heart. Previous studies have focused on disturbances in Ca^{2+} release and reuptake from the sarcoplasmic reticulum (SR), whereas functional studies of the cardiac $\text{Na}^{+}\text{-Ca}^{2+}$ exchanger, another important determinant of myocyte homeostasis, are lacking for the failing **human** heart. Using a cardiac $\text{Na}^{+}\text{-Ca}^{2+}$ exchanger **cDNA** recently cloned from a guinea pig **cDNA** library, the authors investigated the gene expression of the cardiac $\text{Na}^{+}\text{-Ca}^{2+}$ exchanger in relation to the SR $\text{Ca}^{2+}\text{-ATPase}$. Expression of both genes was quantified in left ventricular myocardium from 24 failing **human** cardiac explants and 7 control heart samples in relation to **.beta.-myosin heavy chain** mRNA by slot blot anal. Compared with patients with nonfailing hearts, patients with dilated cardiomyopathy (DCM, n=13) showed a 55% increase in $\text{Na}^{+}\text{-Ca}^{2+}$ exchanger mRNA levels ($P<.05$ vs. control value) and a 41% increase in patients with coronary artery disease (CAD, n=11). In the same hearts, SR $\text{Ca}^{2+}\text{-ATPase}$ mRNA levels were decreased by 50% in DCM and by 45% in CAD ($P<.05$ for both vs. control value). There was a pos. correlation between $\text{Na}^{+}\text{-Ca}^{2+}$ exchanger and SR $\text{Ca}^{2+}\text{-ATPase}$ mRNA levels both in normal and failing **human** hearts, albeit with different slopes and intercepts of the regression line. The $\text{Na}^{+}\text{-Ca}^{2+}$ exchanger protein levels as assessed by Western blot anal. and normalized to **.beta.-myosin heavy chain** protein were increased in DCM and CAD ($P<.05$ and $P<.01$ vs. control value, resp.), whereas SR $\text{Ca}^{2+}\text{-ATPase}$ protein levels were reduced ($P<.05$ for both groups vs. control values). Thus, the $\text{Na}^{+}\text{-Ca}^{2+}$ exchanger gene expression is enhanced in failing **human** hearts and may, in part, compensate for the depressed SR function with regard to diastolic Ca^{2+} removal.

L7 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1993:119264 HCAPLUS
DOCUMENT NUMBER: 118:119264
TITLE: Primary structure of the hinge region in adult chicken cardiac myosin subfragment-2
AUTHOR(S): Watanabe, Bunji; Tanigawa, Mihoko
CORPORATE SOURCE: Sch. Allied Med. Sci., Nagasaki Univ., Nagasaki, Japan
SOURCE: Biological Chemistry Hoppe-Seyler (1993),
374(1), 27-35
CODEN: BCHSEI; ISSN: 0177-3593

DOCUMENT TYPE: Journal
LANGUAGE: English

AB The complete amino-acid sequence of the hinge region in the subfragment-2 (S-2) derived from adult chicken cardiac ventricular muscle myosin has been detd. by direct protein sequencing. The entire amino-acid sequence of this hinge composed of 143 residues was established by structural anal. of CNBr peptides, lysyl and arginyl endopeptidase peptides of carboxymethylated S-2. By sequence comparison with the corresponding region of the same chicken cardiac myosin which was recently deduced from its **cDNA** eight amino-acid differences were recognized. Comparing the sequence of this hinge with those of other cardiac myosins such as rat **.alpha.-** and **.beta.-myosin heavy chains** (MHC), rabbit **.alpha.-MHC** and **human .alpha.-** and **.beta.-MHCs** relatively lower degrees of sequence identities, namely 74.8%, 77.6%, 76.1%, 75.5% and 75.5%, are obsd. On the other hand, more than 89.5% sequence identities are shown among these mammalian cardiac myosins. These results indicate that avian cardiac MHC has diverged earlier than mammalian cardiac myosin has diverged to **.alpha.-** and **.beta.-MHC**. Amino-acid substitutions in this hinge region form a cluster on the C-terminal sequence region. On the contrary, in the N-terminal portion, completely conserved segments are obsd., suggesting that these regions may contribute to the myosin **ATPase** activity and muscle contraction.

L7 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1985:573217 HCAPLUS
DOCUMENT NUMBER: 103:173217
TITLE: Isolation of genomic clones coding for the heavy chains of two human cardiac myosins
AUTHOR(S): Catanzaro, Daniel F.; O'Connell, Anita M.; Morris, Brian J.
CORPORATE SOURCE: Dep. Physiol., Univ. Sydney, Sydney, 2006, Australia
SOURCE: Clinical and Experimental Pharmacology and Physiology (1985), 12(3), 295-7
CODEN: CEXPB9; ISSN: 0305-1870
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A 14-kilobase-pair (kb) **DNA** clone (.lambda.HCMHC8) was isolated from a **human** genomic library by hybridization with a **cDNA** for a rabbit cardiac **myosin heavy chain**. Clone .lambda.HCMHC8 hybridized to RNA isolated from cardiac, but not skeletal, muscle and formed heteroduplexes with a genomic clone for the fast type of rabbit cardiac **myosin heavy chain**. Clone .lambda.HCMHC8 represented at least the 3' half of the genome and contained >11 exons which together spanned 4 kb of the coding region (estd. to be 6 kb). Probes made from .lambda.HCMHC8 were used to rescreen the library to isolate overlapping clones and extend the sequence (estd. to be .apprx.25 kb for the whole gene, including introns). A clone with a different restriction map was isolated, which suggested that **man**, like rat and rabbit, has 2 cardiac **myosin heavy chain** genes. These genes may code for proteins with different **ATPase** activities and may be expressed in different proportions in different cardiac states, including hypertension.

L7 ANSWER 7 OF 7 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1983:85243 HCAPLUS
DOCUMENT NUMBER: 98:85243
TITLE: Composition of subunits and enzymic properties of human and rabbit skeletal muscle myosins
AUTHOR(S): Printsev, M. D.
CORPORATE SOURCE: S. M. Kirov Army Med. Coll., Leningrad, USSR
SOURCE: Ukrainskii Biokhimicheskii Zhurnal (1978-1999) (1983), 55(1), 8-12
CODEN: UBZHD4; ISSN: 0201-8470
DOCUMENT TYPE: Journal
LANGUAGE: Russian

AB **Human** skeletal muscle myosin was more difficult to purify from actin and **nucleic acid** contaminants and was more labile than rabbit muscle myosin. Like the rabbit myosin **ATPase** (EC 3.6.1.3) activity, that of **human** muscle showed max. activity at Ca²⁺ concns. of 8.5-12.8 mM and pH 9.86. At pH 7.6, the **human** myosin **ATPase** activity fluctuated substantially in different expts. but was much lower (mean of 0.17 .mu.mol phosphate/mg protein/min (units)) than that of rabbit (mean of 0.33 units). The **human** myosin prepn. possessed noticeable cholinesterase (EC 3.1.1.8) (I) activity (1.1-1.6 .mu.mol acetylcholine/mg protein/h), whereas rabbit myosin prepn. contained little or no I activity. Evidently, **human** myosin binds I mols. to form fairly stable complexes. During 4M urea-6% polyacrylamide gel electrophoresis (PAGE) of both rabbit and **human** myosins, much of the material, including nondissocd. myosin and **myosin heavy chains** did not enter the gel, but the material which did was sepd. into 7 bands. During SDS-PAGE, both myosins gave 3 light chain bands with mol. wts. of 13,310 (LC3), 18,190 (LC2), and 24,390 (LC1). However, the relative contents of these bands were different, with those for LC1, LC2, and LC3 being 38.9, 52.0, and 9.0%, resp., for **human** and 24.0, 58.3, and 17.7%, resp., for rabbit.